VERIFICATION OF TRANSLATION

Re: Japanese Patent Application NO. 2004-114476

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hereby declare that I am the translator of the document attached and certify that the following is true translation to the best of my knowledge and belief.

Signature of translator 
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[Document Name] CLAIMS

[Claim 1] A reagent composition having a sulfite concentration of more than 6.2 M.

[Claim 2] The reagent composition according to claim 1 having a sulfite concentration from more than 6.2 M to 10 M or less.

[Claim 3] The sulfite reagent composition according to claim 1 or 2, wherein the sulfite is a mixture of ammonium salt and sodium salt.

[Claim 4] The reagent composition according to claim 3, the composition being a mixture of ammonium sulfite, ammonium bisulfite and sodium bisulfite.

[Claim 5] The reagent composition according to any one of claims 1 to 4, the composition having a pH of 5.0 to 5.6.

[Claim 6] A method for deaminating a nucleic acid in a sample DNA, the method comprising the following steps of (a) denaturing a double-stranded DNA into single-stranded DNAs (this step can be skipped when a single-stranded DNA is used), (b) treating the sample in (a), under acidic conditions, at a sulfite concentration of more than 5 M, and (c) treating the sample with an alkali.

[Claim 7] The method for deamination according to claim 6, wherein the nucleic acid to be deaminated is cytosine.

[Claim 8] The method for deaminating a nucleic acid according to claim 6, wherein the sulfite concentration in step (b) is from 6 M to 10 M.

[Claim 9] The method for deaminating a nucleic acid according to claim 6, wherein the pH in step (b) is from 5.0 to 5.6.

[Claim 10] The method for deaminating a nucleic acid according to any of claims 6 to 9, wherein the treatment in step (B) is carried out at 70 °C or higher for less than 1 hour.

[Claim 11] A method for detecting methylated DNA comprising, after treating a sample DNA by any method of claims 6 to 10, detecting the locations of 5-methylcytosine and uracil in

the sample.

[Claim 12] The method for detecting methylated DNA according to claim 11 comprising, after treating a sample DNA by any method of claims 6 to 10, amplifying the sample, and detecting the locations of cytosine and thymine in the amplified sample.

[Claim 13] The method for detecting methylated DNA according to claim 12, wherein the method for detecting the locations of cytosine and thymine is carried out by using base sequence determination, a DNA tip, or a restriction enzyme.

[Claim 14] The method for detecting methylated DNA according to claim 11, the method comprising treating a sample DNA by any method of claims 6 to 10, amplifying the sample using at least one primer that can amplify a nucleic acid when cytosine in the sample DNA is converted to uracil and at least one primer that can amplify a nucleic acid when cytosine is not converted to uracil, and identification was performed based on the presence or absence of amplification.

[Claim 15] A kit for carrying out the deamination method of claim 6, the kit for deaminating a nucleic acid in a sample DNA comprising a reagent composition capable of maintaining acidic conditions and a sulfite concentration of more than 5 M in the step of treating a single-stranded DNA, under acidic conditions, at a sulfite concentration of more than 5 M.

[Claim 16] A kit for carrying out the detection method of methylated DNA of claim 11, the kit comprising a reagent composition capable of maintaining acidic conditions and a sulfite concentration of more than 5 M in the step of treating a single-stranded DNA, under acidic conditions, at a sulfite concentration of more than 5 M.

DOCUMENT NAME SPECIFICATION

TITLE OF THE INVENTION

REAGENT COMPOSITION FOR DEAMINATING NUCLEIC ACID AND METHOD OF DETECTING METHYLATED DNA

TECHNICAL FIELD

[0001]

The present invention relates to a method for deaminating a nucleic acid, and a method, comprising the deamination method, for detecting methylated DNA in a sample DNA.

BACKGROUND ART

[0002]

It is known that methylation of genome regulates the expression of genes in a eukaryote. Therefore, it is very important to identify methylated DNA as genetic information. In particular, 5-methylcytosine is the most frequently modified base in the genome of a eukaryote, and it is also known that aberration thereof causes a genetic disease or a cancer. However, since 5-methylcytosine has the same base sequence as cytosine, it cannot be identified by sequence determination or PCR.

[0003]

The method that is used most frequently as a means for solving this problem is a method for deaminating cytosine by reacting genomic DNA with a sulfite, and converting it to uracil by alkaline hydrolysis (uracil corresponds to thymine in subsequent base-pair behavior.) 5-Methylcytosine is not affected under such conditions (e.g., see Non-patent Document 1). Therefore, if base sequence is determined after such a treatment is performed, only the location of 5-methylcytosine will be determined as cytosine, whereby the location of 5-methylcytosine can be determined (e.g., see Non-patent Document 2).

[Non-patent Document 1] Hayatsu et al., Biochemistry, VOL 9, P2858-2865(1970)

[Non-patent Document 2] Formmer et al., Proc-Natl, Acad Sci. USA, VOL89, P1827-1831 (1992)

[0004]

The reaction conditions of DNA with a sulfite are generally set at 50 °C. for 12 to 16 hours in 4.9 M sodium bisulfite solution (pH 5) (e.g. see Non-patent Document 3). Such a reaction condition was one of the causes why the detection of 5-methylcytosine cannot be rapidly carried out, and the development of a rapid reaction method was needed.

[Non-patent Document 3] Eads et al., Methods in Molecular Biology, VOL.200 P71-85 (2002)

DISCLOSURE OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0005]

An object of the present invention is to enable rapid deamination reaction of a nucleic acid and detection of methylated DNA in DNA in a short time. More specifically, it is to enable rapid deamination reaction of cytosine and detection of methylated cytosine in DNA in a short time.

MEANS FOR SOLVING THE PROBLEMS

[0006]

In order to attain the object described above, the present inventors conducted intensive investigations. As a result, the inventors found that a sulfite solution with a high sulfite concentration can be prepared by mixing different species of sulfite; and the deamination reaction of cytosine proceeds in an extremely short time when DNA is reacted with the highly concentrated sulfite, whereby the present invention has been accomplished. More specifically, the present invention provides: (1) a reagent composition having a high sulfite concentration, (2) a method for rapidly deaminating cytosine wherein DNA is reacted with a sulfite solution having a high sulfite concentration, and (3) a method for detecting methylated DNA

comprising after reacting DNA to the sulfite solution having a high sulfite concentration, detecting the locations of 5-methylcytosine and uracil in the sample.

EFFECTS OF THE INVENTION

[0007]

When the deamination reaction of DNA is carried out by using the sulfite reagent composition having a high sulfite concentration of the present invention, there are advantages in that the deamination reaction that proceeds in a short time, thereby rapidly detecting methylated DNA. Further, since the reaction proceeds in a short time, there are advantages in that hydroquinone used to prevent DNA degeneration is not required, and hence DNA is rarely degenerated (damaged).

THE BEST MODE FOR CARRYING OUT THE INVENTION

[0008]

Hereunder, the present invention is described in detail. One of the aspects of the present invention is a sulfite composition having a high sulfite concentration.

[0009]

The sulfurous acid in the present invention refers to H_2SO_3, HSO_3^-, SO_3^{--}, and the like, when represented by a chemical formula. Under an acidic condition, which is a preferred embodiment of the present invention, almost all are present as a bisulfite ion (HSO_3^-).

[0010]

If the sulfite concentration in the sulfite reagent composition having a high sulfite concentration of the present invention is too low, the deamination reaction rate tends to decrease. Therefore, the sulfite concentration is preferably 6.2 M or more, and more preferably 8 M or more. On the other hand, if the concentration is too high, a crystal will be easily formed. It is therefore preferable that the sulfite concentration is 10 M or less.

It is preferred that the pH of the sulfite reagent composition having a high sulfite concentration of the present invention be substantially the same as the optimal pH for deamination reaction. The higher a sulfite concentration, the faster the deamination reaction tends to proceed. For this reason, it is desirable to avoid adding unnecessary solutions other than a sample nucleic acid and the reagent having a high sulfite concentration. Therefore, the most preferable composition has a sulfite concentration of from 8 M to 10 M and a pH of from 5.0 to 5.6.

[0011]

A sulfite reagent composition having such a high sulfite concentration can be prepared by mixing different species of sulfite.

Examples of sulfite species to be mixed include sodium bisulfite, sodium sulfite, ammonium sulfite, ammonium bisulfite, potassium sulfite, and the like. Among these, for reasons of solubility and pH after the preparation, it is preferable to combine ammonium bisulfite , ammonium sulphite, and sodium bisulfite. The sulfite reagent composition is preferably prepared by adding sodium bisulfite powder and ammonium sulfite powder to an ammonium bisulfite solution (available only in the solution form), and heating the mixture at 70 °C for 5 to 10 minutes. The sulfite reagent composition with a high sulfite concentration, which was not obtainable using a single sulfite, can be thus obtained.

[0012]

One of the aspects of the present invention is a method for deaminating a nucleic acid.

The deamination of nucleic acid includes the steps of treating a single-stranded nucleic acid, under acidic conditions, at a high sulfite concentration, and further treating the nucleic acid with alkali. When a sample is double-stranded DNA, a further step of denaturing it to a single-stranded DNA is included.

In this method, the sulfite reagent composition having

a high sulfite concentration of the present invention described above may be used in the step of treating a single-stranded nucleic acid, under acidic conditions, at a high sulfite concentration.

[0013]

The conditions of the DNA deamination reaction of the present invention preferably has a sulfite concentration of more than 7 M, and a pH of 5.0 to 5.6. When a sulfite concentration is low, the deamination reaction rate slows down. Either a too low or too high pH will cause the deamination ratio to decrease. The reaction is preferably carried out at 70 to 90 °C for 10 to 20 minutes. If the reaction time is too short, deamination of cytosine will be insufficient, whereas if the reaction time is too long, damages to a nucleic acid such as degeneration, etc., will occur.

[0014]

One of the aspects of the present invention is a method for detecting methylated DNA from the nucleic acid after the deamination reaction described above.

Preferable example is to rapidly perform the deamination reaction of cytosine and detect methylated cytosine in DNA in a short time.

Examples of usable methods in the present invention for detecting methylated DNA after the deamination reaction include: a method for detecting the locations of cytosine and thymine by base sequence determination after performing PCR; a method that uses a DNA chip on which a probe hybridizing to a sample in the case where cytosine is converted to thymine and a probe hybridizing to a sample in the case where cytosine is not converted to thymine have been immobilized; a method by which the detection is performed based on the presence or absence of a DNA fragment by using a restriction enzyme which digests DNA and/or a restriction enzyme which does not digest DNA by converting cytosine to thymine; a method wherein DNA in a sample is amplified using at least one primer that can amplify a nucleic

acid in the case where cytosine in the sample DNA is converted to uracil and at least one primer that can amplify a nucleic acid in the case where cytosine is not converted to uracil, and the detection is performed based on the presence or absence of amplification. The method that employs PCR is preferable, but is not limited thereto.

EXAMPLE

[0015]

The present invention is not limited to the following Examples.

[Example 1] Measurement of sulfite concentration

The measurement of a sulfite concentration was carried out by utilizing the fact that sulfur dioxide is generated from a sulfite in a solution of hydrochloric acid and an absorbance at 276 nm (A_{276}) changes depending on the amount of generated sulfur dioxide. To a cuvette for measuring absorbance (1 x 1 x 4 cm, manufactured by Hitachi Ltd.), 3 ml of 0.1 N hydrochloric acid (manufactured by Wako Pure chemical Co., Ltd.) was added. To the cuvette, 30 µl of a sample diluted with distilled water was added, the cuvette was covered with a parafilm, and inverted 3 times to mix the solution. Then, the absorbance at 276 nm was measured with a spectrophotometer (Model U-2800, manufactured by Hitachi Ltd.). The solutions of sodium sulfite (manufactured by Wako Pure Chemical Co., Ltd.) diluted from 0.2 to 3 mM were used as standard solutions and the absorbance thereof was measured in the same manner. The sulfite concentration in a sample was then calculated from the absorbance values of the standard solutions and the sample. When the sulfite concentration of a commercial 50% ammonium bisulfite (manufactured by Wako Pure Chemical Co., Ltd.) was measured by this method, it was from 6.0 M to 6.2 M.

[0016]

[Example 2] Measurement of solubility

The solubilities of sodium bisulfite, sodium sulfite and ammonium sulfite monohydrate (all manufactured by Wako Pure

Chemical Co., Ltd.) were measured. At 30 °C and 70 °C, each reagent was added to 10 ml of distilled water until the reagent was no longer dissolved, and the mass, volume and pH at that time were measured. In addition, the sulfite concentration of each solution was measured by the method described in Example 1. Table 1 shows the results. In the table, the calculated value is that calculated from the mass and the molecular weight of each dissolved sulfite, and the measured value is that measured in accordance with the method described in Example 1. The solubility at 70 °C was 5.9 M for sodium bisulfite, 2.1 M for sodium sulfite and 4.6 M for ammonium sulfite monohydrate.

[0017]

[Table 1]

Reagent	Temp.	Concentration			pH
		g/ml	M		
			Calculated Value	Measured Value	
Sodium Bisulfite	30 °C	0.49	5.2	5.0	4.4
	70 °C	0.61	6.5	5.9	4.5
Sodium Sulfite	30 °C	0.20	1.6	1.5	10.3
	70 °C	0.26	2.1	2.1	10.5
Ammonium Sulfite Monohydrate	30 °C	0.51	3.5	3.5	8.5
	70 °C	0.67	4.6	4.6	8.2

[0018]

[Example 3] Preparation of highly concentrated sulfite solution

To 5.0 ml of ammonium bisulfite solution, 2.08 g of sodium bisulfite and 0.67 g of ammonium sulfite were added and stirred at 70 °C for 5 minutes to dissolve them. The pH of the obtained solution was 5.4. The sulfite solution having a sulfite

concentration of 10 M was then obtained. The pH and the sulfite concentration of this solution did not change at 70 °C for 4 hours.

[0019]

[Example 4] Deamination reaction rate of 2'-deoxycytidine and 5-methyl-2'-deoxycytidine by sulfite treatment

The quantitative determination of deamination reaction product was performed in accordance with the method described in Non-patent Document 4. 2'-Deoxycytidine and 5-methyl-2'-deoxycytidine (produced by Sigma Co., Ltd.) were dissolved in distilled water to give a concentration of 0.2 M. To 25 µl of the 2'-dioxycytidine solution, 250 µl of 5.9 M sodium bisulfite solution prepared in Example 1 (the final reaction concentration: 5.9 x 250 ÷ (250 + 25) ≒ about 5.3 M), or 250 µl of 10 M sodium bisulfite-ammonium solution prepared in Example 3 (the final reaction concentration: 10.0 x 250 ÷ (250 + 25) ≒ about 9.0 M) was added, and a treatment was carried out for 0 to 10 minutes, and 500 µl of chilled water was added to stop the reaction. 75 µl of the reaction solution was mixed with 5 ml of 0.2 M sodium phosphate buffer (pH 7.2) and the mixture was left at room temperature for 40 minutes. Then, the absorbance at 270 nm was measured with a spectrophotometer (Model U-2800, manufactured by Hitachi Ltd.). The absorbance of an unreacted sample was 0.8. The absorbance of only 9 M sodium bisulfite-ammonium solution was 0.05. The absorbance of the unreacted sample was defined as 100%, and the deamination reaction product was quantified by the decrease in the absorbance of a reacted sample. With regard to 5-methyl-2'-deoxycytidine, the absorbance at 277 nm was measured.

[Non-patent Document 4] Sono et al., J. Am. Chem. Soc., VOL96, P4745-4749 (1973)

[0020]

The results of deamination reaction are shown in FIG. 1. The half-life (t 1/2) of deoxycytidine, when the reaction was carried out at 70 °C and pH 5.4, was 3 minutes in the case of using 5.3 M sodium bisulfite solution, whereas it was 1.8 minutes in the case of using the 9.0 M sodium bisulfite-ammonium solution.

The half-life of deoxycytidine at 5.3 M/the half-life of deoxycytidine at 9.0 M is 1.7, which agrees with the ratio of concentrations (9.0/5.3). In other words, it was indicated that the rate of deamination reaction depends on the sulfite concentration. In addition, 10 M sodium bisulfite-ammonium solution was serially diluted and a treatment of deamination reaction was carried out at a concentration of 2 M to 9 M. As a result, it was found that the deamination reaction depends on the sulfite concentration. The deamination ratio of 5-methyl-2'-deoxycytidine in the 9.0 M sodium bisulfite-ammonium solution was 16% when the reaction was carried out at 70 °C for 10 minutes, and 23% at 90 °C for 10 minutes.

[0021]

[Example 5] Temperature dependency of deamination reaction

The half-life of deoxycytidine in the case of performing a treatment with 9.0 M sodium bisulfite-ammonium solution (pH 5.4) at 90 °C, 50 °C and 37 °C were measured by the same method as in Example 4. As a result, they were 1 minute or less, 5 minutes and 17 minutes, respectively.

[0022]

[Example 6] Measurement of time for 100% deamination

The time taken to completely convert 2'-deoxycytidine to 2'-deoxyuridine was measured. To 25 µl of 0.2 M 2'-dexocytidine, 250 µl of 10 M sodium bisulfite-ammonium solution prepared in Example 3 (reaction final concentration: 9 M) was added. The mixture was treated for various times, and subsequently treated with alkali, whereby the deamination reaction product was converted to 2'-deoxyuridine.

10 µl of a sample was subjected to the HPLC analysis described below, and the amounts of 2'-deoxycytidine and 2'-deoxyuridine were measured.

[0023]

Ultrasphere ODS 4.6 mm x 25 cm column (manufactured by Beckman-Coulter Co.) was connected to an HPLC analysis sytem

(manufactured by Hitachi Ltd.). Buffer A (100 mM potassium phosphate buffer (pH 7.0)) and Buffer B (90% methanol, 1 mM potassium phosphate buffer (pH 7.0)) were prepared. In the program of the HPLC system, the flow rate was set at 0.7 ml/min, and the buffer concentration profile was set to 100% A: 0 min, 100% A: 5 min, 85% A: 25 min, 55% A: 35 min, and 0% A: 60 min. The elution times under the condition were 19 minutes for 2'-deoxycytidine, 22 minutes for 2'-deoxyuridine, 25 minutes for 5-methyl-2'-deoxycytidine, 26 minutes for 2'-deoxyguanosine, 28 minutes for thymidine and 32 minutes for 2'-deoxyadenosine. The concentration was calculated from the area of a chart.

[0024]

It was found that, in the case where deamination treatment was performed by using 9.0 M sodium bisulfite-ammonium solution (pH 5.4), the time taken to completely (100%) convert 2'-deoxycytidine to 2'-deoxyuridine was 30 minutes at 70 °C and 8 minutes at 90 °C.

[0025]

[Example 7] pH Dependability of deamination reaction

In 50% ammonium bisulfite solution, sodium bisulfite and sodium sulfite were dissolved at a given ratio, and 7 M sulfite solution at a pH of 4.0 to 6.0 was prepared.

By using this solution, the deamination ratio of 2-deoxycytidine was measured by the same method described in Example 4. The reaction time was set to 5 minutes and the temperature was set to 60 °C. As shown in FIG. 2, an optimal pH was 5.0 to 5.6.

[0026]

[Example 8] Deamination reaction of genomic DNA

Salmon testis DNA (manufactured by Sigma Co.) was dissolved in sterile water to a concentration of 1.6 mg/ml. To 50 μl of this solution, 5 μl of 3 N sodium hydroxide (manufactured by Wako Pure Chemical Co., Ltd.) was added, a treatment was carried out at 30 °C for 30 minutes, whereby a double-stranded DNA was denatured into single-stranded DNAs. Subsequently, to the obtained solution, 550 μl of 10 M ammonium-sodium sulfite

solution (pH 5.4) was added and mixed well. Then, reaction was carried out at 90 °C for 10 minutes (the final concentration of sulfite was 9 M). Subsequently, the reaction solution was applied to a Sephadex G-50 column (ϕ15 x 40 mm, BioRad Econopack 10, manufactured by BioRad Co.), which had been buffered with TE buffer (10 mM Tris-HCl (pH 8), 1 mM EDTA), and a desalting operation was carried out. A DNA fraction was collected by UV monitoring, chilled ethanol (manufactured by Wako Pure Chemical Co., Ltd.) 2.5 times the volume of the collected DNA fraction and 3 M sodium acetate (pH 5.2) one-tenth the volume of the collected DNA fraction were added to precipitate DNA. After the precipitated DNA was separated and recovered by centrifugation, it was dissolved in 100 μl of sterile water. To 90 μl of a sample, 11 μl of 2 N sodium hydroxide was added and a treatment was carried out for 10 minutes, whereby cytosine in the DNA was deaminated and converted to uracil.

[0027]

After the treatment, 30 μl of 3 M sodium acetate (pH 5.2), 70 μl of sterile water and 500 μl of chilled ethanol (manufactured by Wako Pure Chemical Co., Ltd.) were added to the solution, and the mixture was left at -20 °C for 1 hour. The precipitated DNA was recovered, and it was dissolved in 40 μl of sterile water. To 30 μl of the DNA solution, 1.5 μl of a reaction buffer (0.1 M magnesium chloride, 0.2 M Tris-HCl (pH 8)) and 10 μg of DNase I (manufactured by Roche Co.) were added, and a treatment was carried out at 37 °C for 2 hours. Then, 0.4 units of snake venom phosphodiesterase (manufactured by Worthington Co. Ltd.) was added, and further reaction was carried out for 90 minutes. Subsequently, 0.2 units of phosphodiesterase and 2 units of alkali phosphatase (manufactured by Promega Inc.) were added and a treatment was carried out for 90 minutes, whereby DNA was digested into nucleosides. The digested products were separated from proteins or unreacted substances by an operation of ethanol precipitation, and then the solution was dried by suction. After the dried product was dissolved in 30 μl of sterile water,

the amount of nucleosides were measured by the HPLC analysis described in Example 6.

[0028]

The charts of the HPLC analyses are shown in FIG. 3, and the ratio of each nucleoside is shown in Table 2. In 9 M ammonium-sodium sulfite solution, the deamination ratio of cytosine (conversion ratio from cytosine to uridine) in genomic DNA when a treatment was carried out at 90 °C for 10 minutes was 99.6%. In addition, the conversion ratio of 5-methylcitosine was 10% or less. Moreover, the conversion of another base was not observed. The reaction times in which the similar deamination ratio was obtained at 70 °C and 37 °C were 16 minutes and 170 minutes, respectively.

[0029]

[Table 2]

	Mol%					
	C	U	mC	G	T	A
Sulfite Treatment	0.08	19.89	1.29	21.56	29.28	27.90
Untreated	20.26	0.04	1.41	22.43	28.67	27.19

In Table 2
C: 2'-deoxycytidine, U: 2'-deoxyuridine, mC: 5-methyl-2'deoxycytidine, G: 2'-deoxyguanosine, T: thymidine, A: 2'-deoxyadenosine.

[0030]

[Example 9] PCR amplification of deaminated DNA

pUC119 (manufactured by Takara Bio Inc.) treated with 1 μg of ScaI restriction enzyme (manufactured by NEB Inc.) was denatured into single-stranded DNAs by treating it in 50 μl of 0.3 N sodium hydroxide solution at 37 °C for 30 minutes. The treated solution was heated for 3 minutes at 70 or 90 °C, to

which 500 μl of 10 M ammonium-sodium sulfite solution (pH 5.4) was added and mixed well. Then, a mineral oil was overlaid, and reaction was carried out at 70 or 90 °C for 5 to 40 minutes. 130 μl of the reaction solution was taken out and mixed with ice-cold sterile water. DNA was purified using Wizard DNA Clean-UP system (manufactured by Promega Inc.) in accordance with the operation manual and dissolved in 90 μl of sterile water. Thereto was added 11 μl of 2 N sodium hydroxide solution, and a treatment was carried out at 37 °C for 10 minutes. By using 10 μg of yeast tRNA (manufactured by Sigma Co., Ltd.) as a carrier, DNA was recovered by an operation of ethanol precipitation and dissolved in 100 μl of TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA).

[0031]

By using 1 μl of this solution as a sample, PCR was performed in a 50 μl reaction system using 2 types of primers (sequences 5'-CGGAATT CTATTGGTTAAAAAATGAG-3' and 5'-AACTGCAGACAT TAACCTATAAAAATA-3') and AmpliTaq DNA polymerase (manufactured by Applied Biosystems Inc.). The cycle condition was 95 °C for 3 minutes followed by 30 cycles of 95 °C for 30 seconds, 57 °C for 30 seconds and 70 °C for 3 minutes. Other conditions were in accordance with the operation manual. After PCR, 1 μl of the sample was analyzed by agarose gel electrophoresis, and the amount of amplification was confirmed.

[0032]

As a result, the amounts of PCR amplification of untreated DNA and the DNA of the sample treated at 70 or 90 °C for 5 minutes to 40 minutes were almost equal. This suggests that the DNA treated with sulfite was not damaged such as cleaved. Further, with regard to the PCR products of the sample treated at 70 °C for 20 minutes and the sample treated at 90 °C for 10 minutes, the nucleotide sequence was determined using BigDyeTM Terminator Cycle Sequencing kit (manufactured by Applied Biosystems Inc.) and the ABI model 3700 autosequencer (manufactured by Applied Biosystems Inc.), and it was found that cytosine was converted to thymine.

[0033]

According to the present invention, as clear in Examples 1 to 9, the deamination reaction of DNA can be carried out in a short time. Further, the present invention enables the rapid detection of methylated DNA.

INDUSTRIAL APPLICABILITY OF THE INVENITON

[0034]

The sulfite reagent with a high sulfite concentration of the present invention enables the deamination reaction of DNA in a short time and rapid detection of methylated DNA, thereby greatly contributing to medical fields such as diagnosis of cancers and genetic diseases, etc.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035]

FIG. 1 is a graph showing the deamination ratio in a sulfite treatment reaction as the remaining amount of cytosine.

FIG. 2 is a graph showing the pH dependency of deamination reaction of the present invention.

FIG. 3 shows graphs showing the HPLC analysis results of a salmon testis DNA sample treated by the deamination method of the present invention.

DOCUMENT NAME

FIG. 1

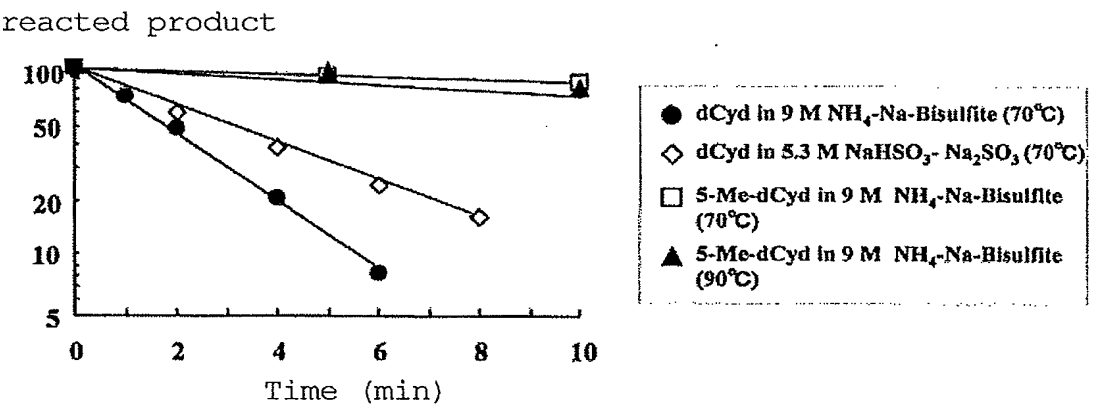


FIG. 2

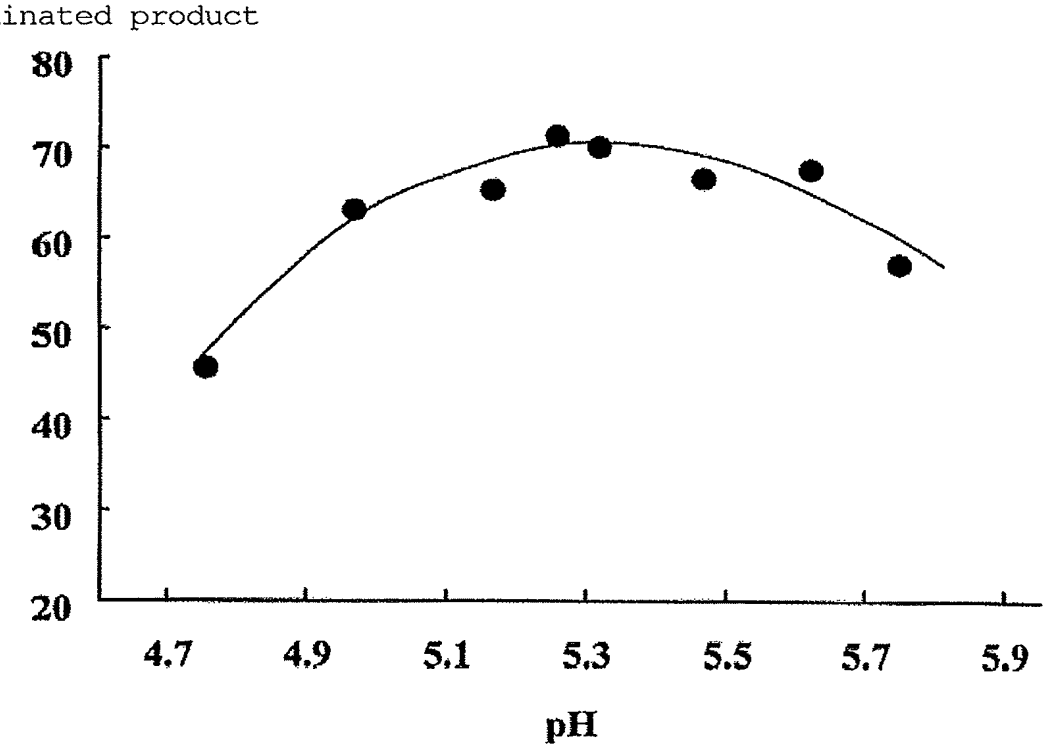
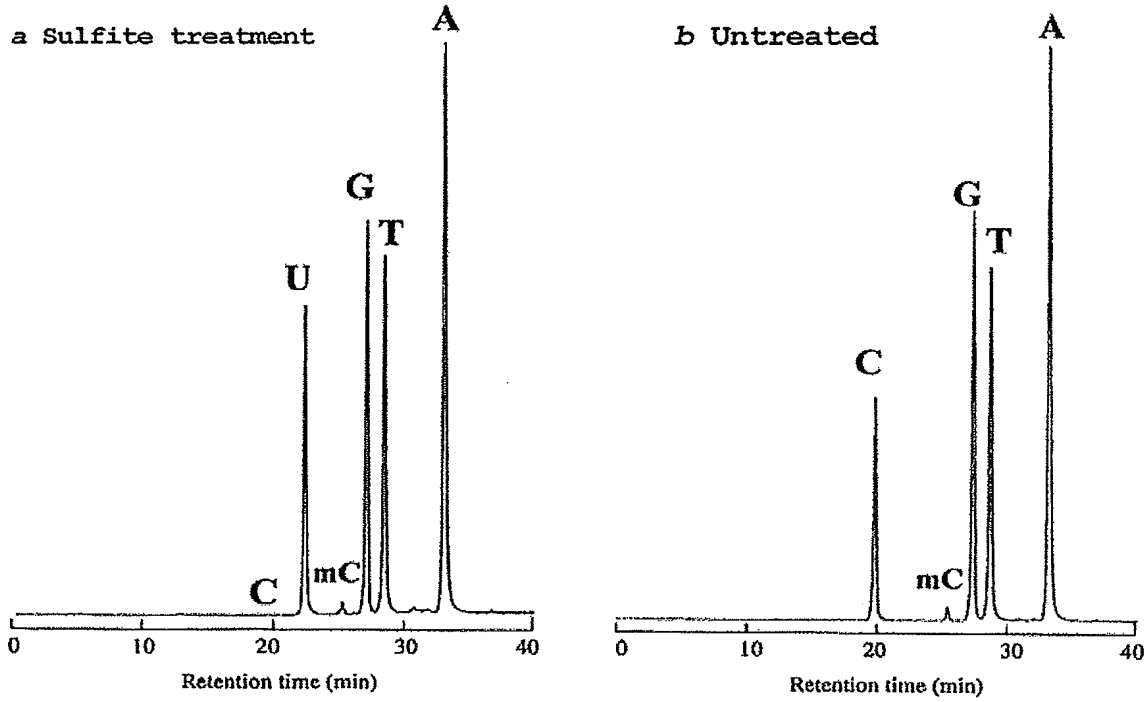


FIG.3



C: 2′-deoxycytidine

U: 2′-deoxyuridine

mC: 5-methyl-2′deoxycytidine

G: 2′-deoxyguanosine

T: thymidine

A: 2′- deoxyadenosine

DOCUMENT NAME ABSTRACT

ABSTRACT

OBJECT To enable deamination reaction of a nucleic acid to proceed rapidly and detection of methylated DNA in DNA in a short time, and more specifically, to enable deamination reaction of cytosine to proceed rapidly and detection of methylated cytosine in DNA in a short time.

MEANS FOR ACHIEVING THE OBJECT To provide a method for detecting methylated DNA comprising: (1) a reagent composition having a high sulfite concentration, (2) a method for rapidly deaminating cytosine wherein DNA is reacted with a sulfite solution having a high sulfite concentration, and (3) after reacting DNA to the sulfite solution having a high sulfite concentration, detecting the locations of 5-methylcytosine and uracil in the sample.